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New distribution records, observations on natural history, and notes on reproduction of the poorly known Sudanese Unicorn Chameleon (Chamaeleonidae: *Trioceros conirostratus*) from Uganda, Africa

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***Abstract.*—We provide data derived from nearly four months of field surveys on the distribution, natural history, and habitat of the poorly known Sudanese Unicorn Chameleon (*Trioceros conirostratus*) from Northern Region, Uganda, Africa. Our study also provides the first description of the reproductive mode and an estimate of the litter size for *T. conirostratus*. Multiple individuals of *T. conirostratus* were detected from mid–high elevation wooded-grassland and closed-forest habitats in six Central Forest Reserves across northeastern Uganda during surveys conducted in 2015 and 2016. *Trioceros conirostratus* is viviparous as evidenced by the presence of well-developed embryos that lacked eggshells in the oviducts. Twelve embryos were present in one of the females. Adult males were smaller on average than adult females. The presence of variously sized juveniles with non-gravid and gravid adult females during surveys at the same site suggested that this species might exhibit asynchronous reproduction. We observed a possible mechanism for predator deterrence in this species from repulsive material stored in temporal pouches. Our results greatly expand the distribution, and significantly add to the knowledge on the reproductive biology and natural history of *T. conirostratus* in Uganda.**

Keywords. Conservation, Central Forest Reserve, East Africa, Karamoja, live-bearing, Sauria, morphology

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Introduction

The Sudanese Unicorn Chameleon (*Trioceros conirostratus* Tilbury, 1998) was described on the basis of a single male specimen collected from 1,050 m at Lomoriti in the Imatong Mountains of southern South Sudan (Tilbury 2010). Since its description in 1998, few observations of this species have been made, including just two verifiable extensions of its geographic range to the Loima Hills (1,400 m) and Mtelo Massif (1,900–2,300 m), both sites in northwestern Kenya (Kořený 2006; Stipala et al. 2011, 2012). The apparent disjunct geographic distribution of *T. conirostratus* prompted speculation on its occurrence at similar elevations between the known localities in southern South Sudan and northwestern Kenya (Stipala 2014a, b; Spawls et al. 2014). However, vouchered specimens have yet to be obtained from potential sites between the documented populations of *T. conirostratus*.

The original description of *T. conirostratus* provided almost no information about its natural or life history, and thus, knowledge of its habitat preferences and ecology is derived from just a few Kenyan samples (Stipala 2014a). For example, this species was originally posited to be a forest specialist because the Imatong Mountains possess large swaths of broad-leafed forest (Tilbury 2010). However, specimens collected from the Mtelo Massif in northwestern Kenya were found on shrubs of a disturbed agricultural area, and the surrounding habitat consisted of xeric-adapted woodland tree species (Stipala 2014a). Reproduction in *T. conirostratus* is unknown, and no empirical investigations have been undertaken to date (Tilbury 2010); however, it has been conjectured to

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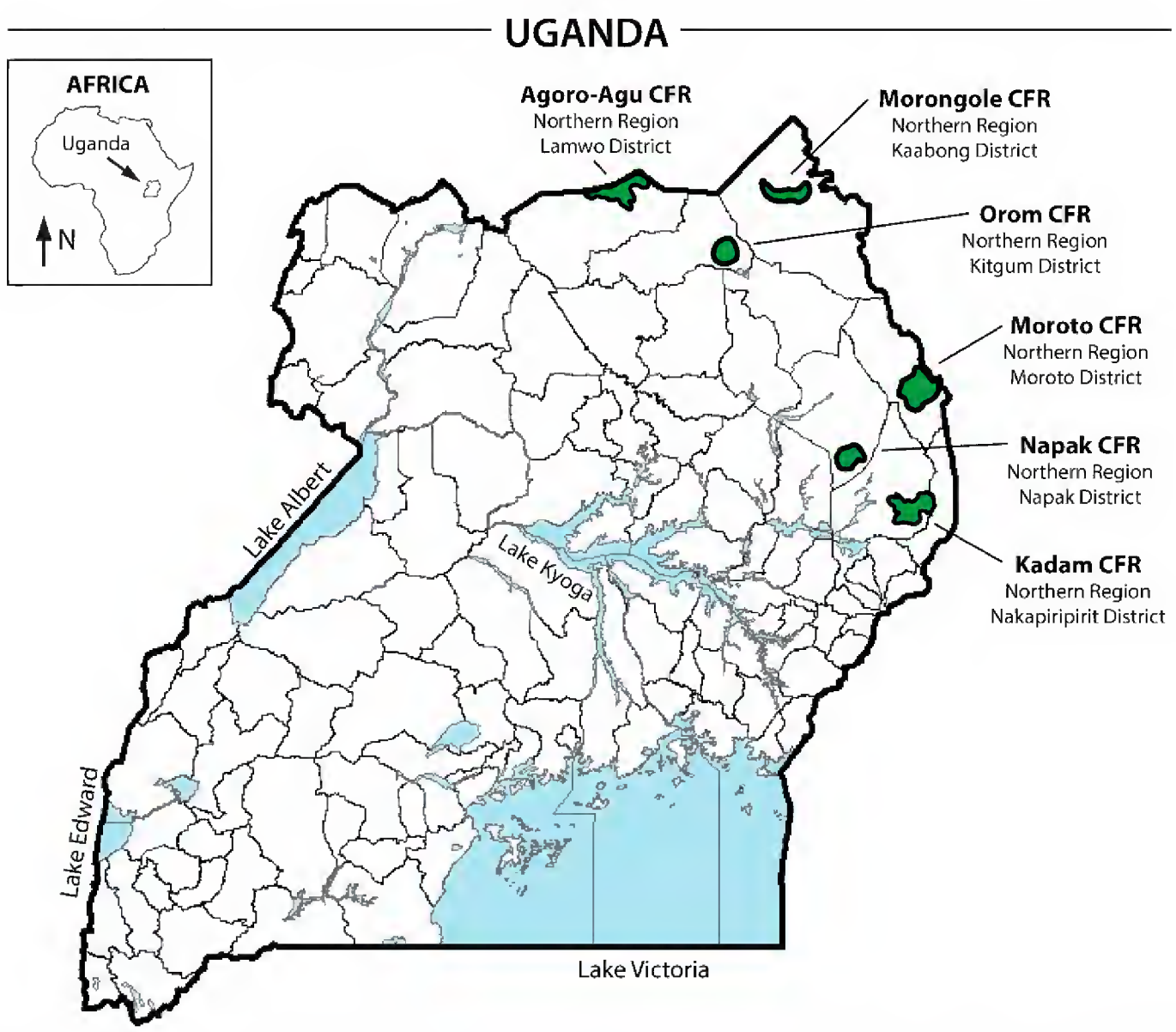

Fig. 1. Locations of Central Forest Reserves (CFR) where the Sudanese Unicorn Chameleon (*Trioceros conirostratus*) was found during surveys from 2015–2016, Northern Region, Uganda.

be viviparous because of its phylogenetic affinities to the live-bearing *T. bitaeniatus* species group (Tilbury and Tolley 2009; Stipala 2014a).

In this report, we provide the first formal records of *T. conirostratus* from six Central Forest Reserves (CFR) in northeastern Uganda that fill critical gaps in the distribution for this species. In addition, we describe the reproductive mode, report on the litter size, and describe a predator deterrent mechanism of this species. Lastly, we present information on body sizes, color patterns, and habitats of *T. conirostratus* from these recently discovered Ugandan populations.

Methods and Materials

We conducted nearly four months of fieldwork in Northern Region, Uganda during May–July 2015 and July–August 2016, throughout which we surveyed the herpetofauna at several protected areas in the region, including six montane CFRs associated with the Karamoja Sub-region. At each CFR, we spent an average of three days and four nights conducting diurnal and nocturnal visual-encounter surveys. Our team, with help from forest reserve managers, Uganda People's Defence Force soldiers, local police, guides, and villagers searched for chameleons in various natural forested and non-forested habitats at each site. Chameleons were most often encountered on sleeping perches at night in various types of vegetation and located with the aid of artificial lights. Fewer chameleons were encountered during diurnal searches. Notes were taken on GPS coordinates, ecology, behavior, date, time, sex, and basic habitat details for each collected specimen. Collected chameleons were humanely euthanized, tissue samples taken from the hind limb or liver and stored in 99% ethanol, and specimens were later fixed in 10% buffered formalin. On completion of each expedition, with permits from the proper authorities (CITES, UWA, UNCST, and UMAAIF), the specimens and tissue samples were transferred to the University of Texas at El Paso's Biodiversity Collections in the United States.

A single gravid female *T. conirostratus* collected from Morongole CFR was set aside for an assessment of its reproductive mode. This female specimen was euthanized, and then its oviducts were removed immediately and placed in a separate vial filled with 10% buffered formalin. Criteria for assessing its reproductive mode were adopted from the literature (Blackburn 1993), and embryonic development was assessed in accord with the Dufaure and Hubert (1961) system (Porter 1972), as modified for chameleons by Andrews (2007).

Results

We found individuals of *T. conirostratus* from several localities within six CFRs across northeastern Uganda: Agoro-Agu, Kadam, Moroto, Morongole, Napak, and Orom (Fig. 1; Table 1). Most individuals were detected in wooded-grasslands and adjacent agricultural fields, and far fewer individuals were found in closed forests. Further, we detected more individuals in semi-disturbed to disturbed grassland-associated areas than in pristine

Table 1. Collection information for the Sudanese Unicorn Chameleon (*Trioceros conirostratus*) during surveys from 2015–2016 in Northern Region, Uganda, Africa. CFR – Central Forest Reserve; + – detected but not collected.

Locality	Coordinates	Elevation (m)	Date	Class (no. individuals)
Agoro-Agu CFR, Lamwo District	N03.81357, E32.94207	2,134	5 July 2015	JV (8)
Kadam CFR, Nakapiripirit District	N01.79864, E34.74146	2,140	31 May 2015	M (1), F (4), JV (9)
Morongole CFR, Kaabong District	N03.80740, E34.02559	2,461	23 July 2016	M (5), F (5), JV (+)
Moroto CFR, Moroto District	N02.51234, E34.70190	1,969	4 June 2015	M (4), F (3), JV (1)
	N02.52847, E34.72872	2,582	5 June 2015	M (2), F (3), JV (4)
Napak CFR, Napak District	N02.04033, E34.27148	1,875	14 July 2016	M (4), F (6), JV (1)
Orom CFR, Kitgum District	N03.40338, E33.55560	1,304	18 July 2016	M (3), F (6), JV (+)

forested habitats. However, we do not know whether the fewer encounters in closed forests were due to actual or apparent rarity of *T. conirostratus* in this habitat type. At most sites during nearly all surveys, we encountered small juveniles, sub-adults, and adults of both sexes (Table 1). Save for the rostral appendage in males, the general appearance of males and females was similar across populations, yet color patterns were variable (Fig. 2–3).

The mean body size of adult males, 66.19 ± 6.09 mm (range 57.87–78.03 mm; n = 14), was significantly smaller than that of adult females, 70.97 ± 6.47 mm (range 57.57–83.58 mm; n = 23) (t = -2.23, df = 35, P = 0.03). The mean tail length of adult males, 66.27 ± 7.8 mm (range 54.51–80.94 mm; n = 14) was about 3 mm longer, but not significantly different, from that of adult females, 63.36 ± 7.95 mm (range 46.33–79.8 mm; n = 23) (t = 1.09, df = 35, P = 0.28). On average, the ratio of male tail length to body size was roughly proportional (mean TL/SVL = 1.0 ± 0.05; range 0.93–1.12; n = 14), whereas this ratio in females was skewed towards body size (mean TL/SVL = 0.89 ± 0.07; range 0.78–1.03; n = 23). Mean body size of juveniles was 30.87 ± 4.97 mm (range 22.13–42.22 mm; n = 21) and mean tail length was 27.22 ± 5.19 mm (range 18.18–39.01 mm; n = 21), with the average ratio of tail length to body size favoring body size in juveniles (mean TL/SVL = 0.88 ± 0.07; range 0.77–0.99; n = 21). The smallest juvenile for which an emerging rostral horn could be seen was 31.7 mm SVL and 23.23 mm TL.

Threatened individuals of *T. conirostratus* (both sexes and juveniles) would open their mouths, yet, rarely would they bite when handled. Rather, it seemed that opening the mouth served a dual purpose: intimidate and expose temporal pouches (i.e., corner of jaw) (Fig. 4). These pouches were often filled with a yellow/orange or brown material that had an astringent odor and was similar in consistency to partially digested food (Fig. 4A). When handled, chameleons would open their mouths, expose the pouches, and extrude this substance. Simultaneously, chameleons would thrash their heads from side to side; an action that often distributed the substance onto the collector's hand (Fig. 4B). The foul-smelling scent from the smeared material remained on the collector for ca. 24 hours post-washing with soap.

We found moderately well-developed embryos within the oviducts of one female specimen (Field no. DFH 975: SVL 66.96 mm and TL 62.23) (Fig. 5). A litter size of 12 embryos was found, with six embryos in each oviduct. The embryos were of stage 35 (*sensu* Andrews 2007), with the following characteristics: a heavily pigmented ocular choroid, an indented external auditory meatus, cervical flexure in the process of disappearing, mandible extends to the tip of the snout, well-developed forelimbs and hindlimbs with fully zygodactylous feet, digits are prominently outlined, and connected by interdigital webbing with slightly concave margins (Fig. 5). The embryos were surrounded by fetal membranes that lay in apposition to the uterine lining, with a chorioallantoic placenta positioned dorsally, and a yolk sac placenta ventrally. A very thin and transparent vestige of an eggshell membrane could sometimes be discerned at the placental interface, yet it showed no trace of calcification. This gravid specimen had a pigmented peritoneum.

Discussion

We documented the presence of *T. conirostratus* from a series of isolated volcanic mountains in Northern Region, Uganda, Africa. The new localities fill in critical gaps for the geographic range of *T. conirostratus*. Importantly, we provide empirical evidence to support a single unsubstantiated report of this species on social media that ostensibly originated from an undisclosed locality in Uganda at 1,800 m (Stipala 2014b). We found that *T. conirostratus* can be found in suitable montane habitat at an elevation range from 1,304 m to 2,582 m in northeastern Uganda. Our field surveys indicate that *T. conirostratus* can occupy both wooded-grassland and closed-forest habitats, and although it was much more common in the former, we do not know if this apparent habitat preference is an artefact of sampling or not. This species seems to tolerate anthropogenic disturbances, largely in the form of slash and burn agricultural farming. In general, male *T. conirostratus* tended to be smaller and have longer tails than females, and this form of sexual dimorphism is shared with members of the *T. bitaeniatus* group and many other chameleons (Tilbury 2010).

Temporal pouch material was recently characterized

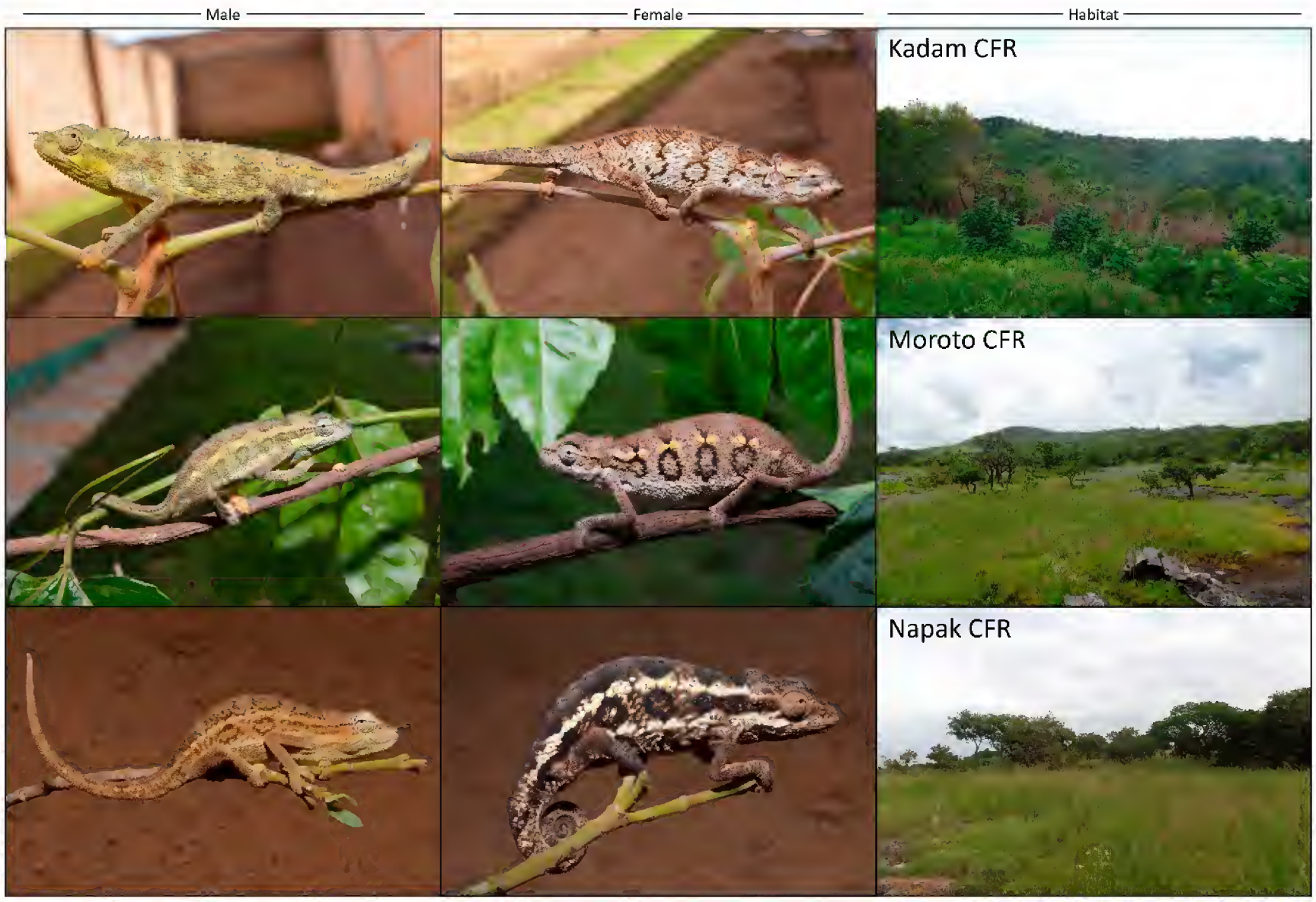

Fig. 2. Representatives of the Sudanese Unicorn Chameleon (*Trioceros conirostratus*) and local habitats from the three "southern" Central Forest Reserves (CFR) surveyed in Northern Region, Uganda, Africa. Male and female from Kadam CFR **(top row)**, male and female from Moroto CFR **(middle row)**, and male and female from Napak CFR **(bottom row)**.

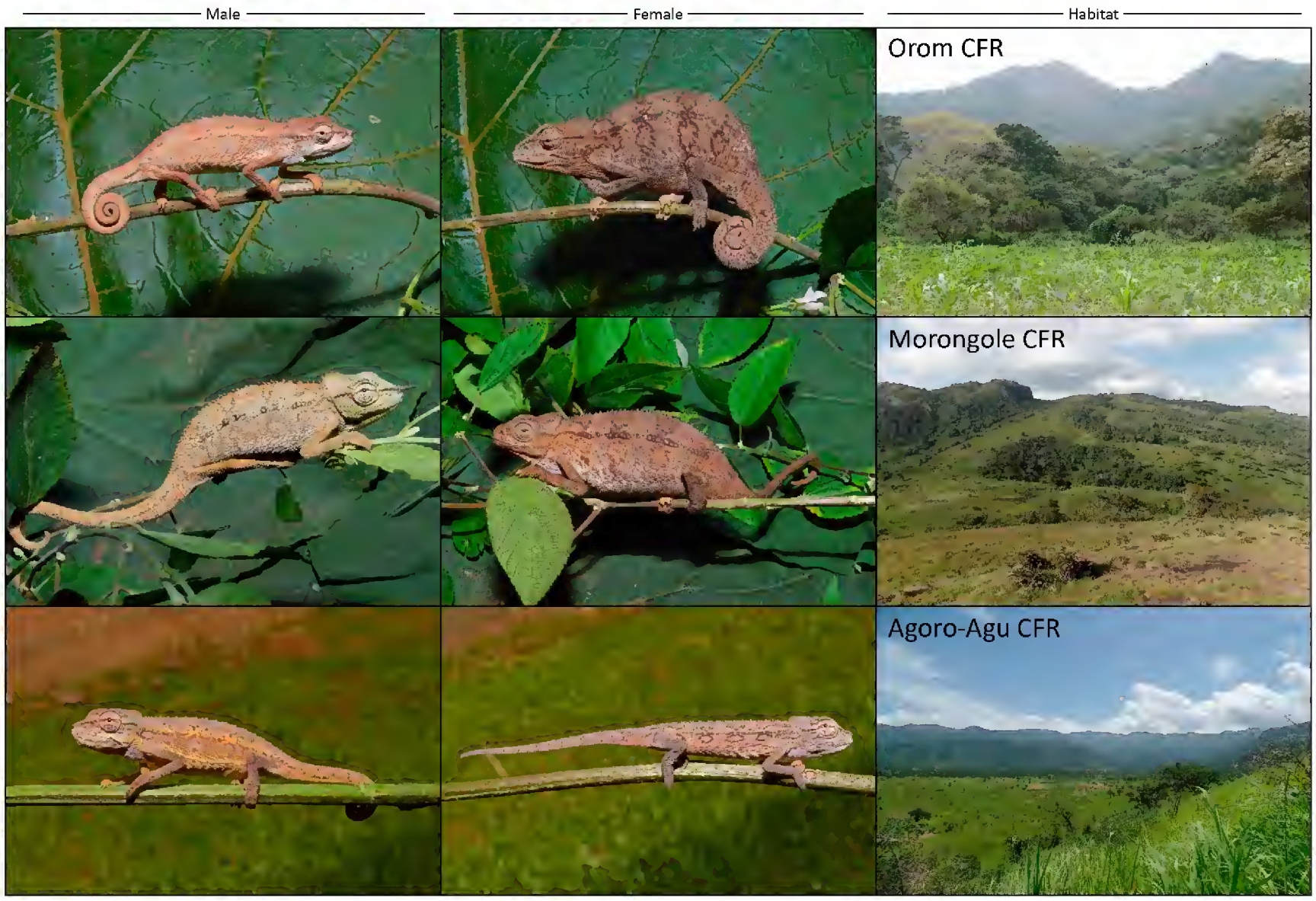

Fig. 3. Representatives of the Sudanese Unicorn Chameleon (*Trioceros conirostratus*) and local habitats from the three "northern" Central Forest Reserves (CFR) surveyed in Northern Region, Uganda, Africa. Sub-adult male and female from Orom CFR **(top row)**, male and female from Morongole CFR **(middle row)**, and juvenile male and juvenile female from Agoro-Agu CFR **(bottom row)**.

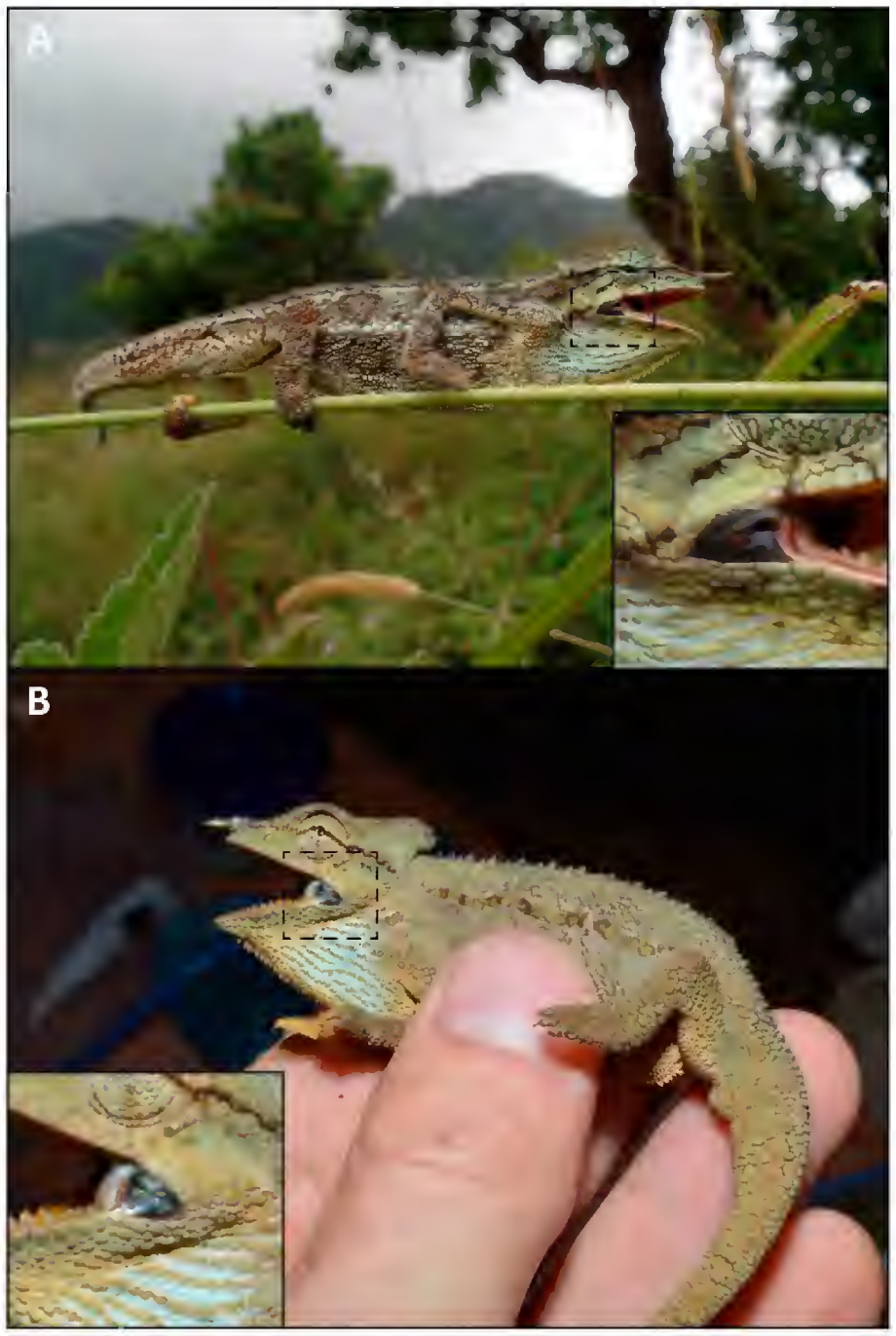

Fig. 4. Examples of Sudanese Unicorn Chameleons (*Trioceros conirostratus*) displaying a threatened posture with the mouth open. **(A)** Threatened male from Morongole Central Forest Reserve (CFR) displaying a temporal pouch filled with brown odiferous material (in-detail); **(B)** Another threatened male from Morongole CFR demonstrating an empty temporal pouch depleted after being handled (in-detail).

from the Jackson's Chameleon (*Trioceros jacksonii*), and it was found to contain volatile and odiferous compounds derived from those found in prey items and/or sloughed skin (Preest et al. 2016). Preest et al. (2016) speculated that this pouch material was involved with insect luring, and while this is likely true, the behavior we observed in *T. conirostratus* seemed more for predator deterrence. We also observed an analogous behavior and foul-smelling substance in three other species of chameleons: *T. ellioti*, *T. johnstoni*, and *Kinyongia xenorhina* from Western Region, Uganda (D.F. Hughes, personal observation).

The reproductive mode of *T. conirostratus* was determined to be viviparous. The embryos were at stage 35, which lies somewhat beyond the stage of oviposition in typical oviparous squamates (Blackburn 1995). In addition, no eggshell was present; rather, dissection revealed a thin, barely-visible vestige of the shell membrane that lacked any trace of calcification. This feature is indicative of viviparity, because in oviparous squamates, an opaque eggshell surrounds embryos of mid-stage and beyond. Given the absence of an eggshell, the chorioallantois and yolk sac formed placentas in conjunction with the uterine

Fig. 5. A developing embryo of the Sudanese Unicorn Chameleon (*Trioceros conirostratus*), removed from a female oviduct. Scale bar = 1 mm.

lining, and their topography conformed to that of typical viviparous lizards (Stewart and Blackburn 2014). The association of viviparity with a pigmented adult peritoneum is consistent with that of other live-bearing *Trioceros* (see Tilbury et al. 2006). The litter size (12 embryos) lies within the range of other viviparous species of the *T. bitaeniatus* group. Reports on litter size are available for several other members of this species group, including *T. ellioti* (4–12 young: Leptien 1989), *T. hoehnelii* (7–18 young: Spawls et al. 2002), and *T. jacksonii* (7–28 young: Spawls et al. 2002).

We encountered small juveniles (< 30 mm SVL), as well as gravid and non-gravid adult females of *T. conirostratus* during the same surveys, and we suggest that this may be indicative of asynchronous reproduction, which is common among live-bearing chameleon species of the South African genus *Bradypodion* (Tolley and Burger 2007; Tolley and Jackson 2014) and other viviparous *Trioceros* species (e.g., *T. bitaeniatus* (Nečas 1999)). More investigation is warranted to determine the ecological variables associated with the timing of reproduction in *T. conirostratus* populations in Uganda.

Conservation

Prior to our surveys in 2015, *T. conirostratus* had not been recorded from Uganda and thus was not included on the country's national checklist of reptiles (Behangana 2015). We consider illegal wildlife trade to be the primary conservation threat facing *T. conirostratus* in Uganda, in large part because this species was considered rare prior to our surveys, and thereby highly coveted by chameleon hobbyists. For instance, a wildlife trafficker had already harvested this species before we first documented its presence in Uganda. In May of 2015, local sources indicated to our team that an international animal dealer purchased 100 live *T. conirostratus* several months prior to our arrival at the Kadam Central Forest Reserve (CFR). We were advised that the chameleons were collected by the indigenous people and purchased

at Uganda shillings 10,000 ($2.70 USD) by a local interpreter on behalf of the trader. The local interpreter in turn received Uganda shillings 80,000 ($21.60 USD) per live chameleon. We discovered that numerous chameleons perished during the collection process and many more died in captivity while amassing the trader's full request.

The IUCN Red List considers *T. conirostratus* as Least Concern (Stipala 2014b), and our surveys generally support this conservation assessment. The populations we encountered in Uganda seemed tolerant of agricultural and fire-based anthropogenic disturbances. However, the conservation of *T. conirostratus* is complicated by the fact that its IUCN assessment was completed prior to our discovery of this species in Uganda. Furthermore, the demand for pet chameleons is often met, in part, by the export of wild animals from several East African countries, including Uganda (Carpenter et al. 2004; Jenkins et al. 2014). According to the CITES Trade Database, over 50,000 live chameleons have been exported from Uganda since 2000, including several species that do not even occur in the country (e.g., *Chamaeleo senegalensis* (Tilbury 2010)). Indeed, because of the general interest for pet chameleons, it is reasonable to think that a trade in this species could develop from Uganda. Based on our surveys, *T. conirostratus* is now formally included in the checklist of Uganda reptiles, which serves as an important first-step towards the sustainable trade in this species (Jenkins et al. 2014). Yet, if collection pressure for this species becomes unsustainable, breeding farms located within the species range may help to mitigate threats from overharvesting while providing a boost to local economies (Otieno 2015).

We do, however, recommend that the governing bodies in Uganda use caution when setting export quotas and licensing international trade permits in *T. conirostratus* for three important reasons: 1) this species is currently known only from protected areas in Uganda; 2) the risk of trading in populations with an unknown conservation status or of dubious origin is high for Uganda; and 3) it has not yet been fully evaluated whether any of these isolated populations represent a cryptic species that could be endemic to Uganda.

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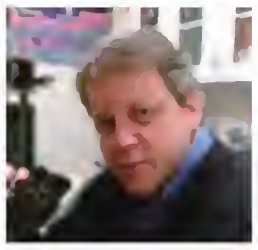

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Lukwago Wilber received his Master's degree in Environment and Natural Resources from Makerere University in 2017. His research focuses on the ecology of reptiles and amphibians in Africa. His forestry background aids him to correlate vegetative changes in forest habitats to herpetological diversity. He currently works with the Uganda National Roads Authority (UNRA) as a Senior Environment Officer and as an Ecologist on all road development projects.

Mathias Behangana received his Ph.D. in Environment and Natural Resources in 2010, and M.Sc. in Zoology in 1997 from Makerere University. He trained as an environmental/wildlife ecologist and has over 23 years of experience in research and monitoring biodiversity with a focus on amphibian and reptilian fauna of Africa. He currently works as the Founding Director for NICE-Planet Limited, a local NGO.